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(54) Title: HYPERSENSITIVE RESPONSE ELICITING DOMAINS AND USE THEREOF

(57) Abstract: The present invention is directed to the structure of an isolated protein or polypeptide which elicits a hypersensi-
tive response in plants as well as an isolated nucleic acid molecule which encodes the hypersensitive response eliciting protein or
polypeptide. This protein or polypeptide has an acid portion linked to an alpha helix or a pair of spaced apart domains comprising
an acidic portion linked to an alpha-helix. This isolated protein or polypeptide and the isolated nucleic acid molecule can be used
to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance to plants. This
can be achieved by applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant
seeds under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or to impart stress
resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a nucleic
acid molecule encoding a hypersensitive response elicitor protein or polypeptide can be provided and the transgenic plants or plants
resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth,
to control insects, and/or to impart stress resistance to plants or plants grown from the plant seeds.

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HYPERSENSITIVE RESPONSE ELICITING DOMAINS AND USE THEREOF

This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 60/212,211, filed on June 16, 2000.

FIELD OF THE INVENTION

The present invention relates to hypersensitive response elicitors and
10 their structure.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular
bacterial growth, symptom development, and disease development in the host plant;
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a
particular type of incompatible interaction occurring, without progressive disease
symptoms. During compatible interactions on host plants, bacterial populations
20 increase dramatically and progressive symptoms occur. During incompatible
interactions, bacterial populations do not increase, and progressive symptoms do not
occur.

The hypersensitive response is a rapid, localized necrosis that is
associated with the active defense of plants against many pathogens (Kiraly, Z.,
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily
30 observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

- "Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause
- 10 physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986);
- 15 Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.
- The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al.,
- 20 "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway
- 25 similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich,
- 30 protein elicitors of the hypersensitive response (He, S.Y., et al. "*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention is a further advance in the effort to identify and characterize hypersensitive response elicitor proteins.

SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated
5 hypersensitive response elicitor protein comprising a pair of spaced apart domains,
with each comprising an acid portion linked to an alpha-helix.

Another embodiment of the present invention relates to an isolated
hypersensitive response elicitor protein comprising an acid portion linked to an alpha-
helix.

10 Nucleic acid molecules encoding either of these proteins as well as
vectors, host cells, transgenic plants, and transgenic plant seeds containing those
nucleic acid molecules are also disclosed.

The protein of the present invention can be used to impart disease
resistance to plants, to enhance plant growth, to control insects, and/or impart stress
15 resistance. This involves applying the protein to plants or plant seeds under
conditions effective to impart disease resistance, to enhance plant growth, to control
insects, and/or impart stress resistance to plants or plants grown from the plant seeds.

As an alternative to applying the protein to plants or plant seeds in
order to impart disease resistance, to enhance plant growth, to control insects on
20 plants, and/or impart stress resistance, transgenic plants or plant seeds can be utilized.
When utilizing transgenic plants, this involves providing a transgenic plant
transformed with a nucleic acid molecule encoding the protein of the present
invention and growing the plant under conditions effective to impart disease
resistance, to enhance plant growth, to control insects, and/or to impart stress
25 resistance to the plants or plants grown from the plant seeds. Alternatively, a
transgenic plant seed transformed with the nucleic acid molecule encoding the protein
of the present invention can be provided and planted in soil. A plant is then
propagated under conditions effective to impart disease resistance, to enhance plant
growth, to control insects, and/or to impart stress resistance to plants or plants grown
30 from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing the construction of a universal expression cassette for a hypersensitive response domain.

5

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to an isolated hypersensitive response elicitor protein comprising a pair of spaced apart domains, with each comprising an acid portion linked to an alpha-helix. The acidic portion is a polypeptide with 10 or more amino acids, is rich in acidic amino acids, and has a pI below 5.0. The acidic portion has a secondary structure in the form of a beta-sheet or a beta-turn. The secondary structure of this unit can also be in an unordered form.

The alpha-helix portion of the present invention is a polypeptide with 10 or more amino acids. Its secondary structure is in the form of a stable alpha-helix.

15

Another embodiment of the present invention relates to an isolated hypersensitive response elicitor protein comprising an acid portion linked to an alpha-helix.

20

Both of these proteins are capable of eliciting a hypersensitive response.

The alpha helix is a common structural motif of proteins in which a linear sequence of amino acid folds into a right-handed helix stabilized by internal hydrogen bonding between backbone atoms.

25

The acidic motif includes a certain combination of amino acids in which a linear sequence with a pI below 5.0 folds into a β sheet, coil, or thin structures but not an alpha helix of secondary structure.

30

The hypersensitive response elicitor polypeptides or proteins according to the present invention can be derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors

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include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

15	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser	1 5 10 15
	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser	20 25 30
20	Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr	35 40 45
	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu	50 55 60
	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	65 70 75 80
25	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	85 90 95
	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	100 105 110
30	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	115 120 125
	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	130 135 140
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	145 150 155 160
35	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	165 170 175

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Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
 180 185 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
 195 200 205

5 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
 210 215 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
 225 230 235 240

10 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
 245 250 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
 260 265 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
 275 280 285

15 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
 290 295 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
 305 310 315 320

20 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
 325 330 335

Asn Ala

25 This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

30 CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG 60
 GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTGCGCGCA ATCCGGCGTC 120
 GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAATCA TGATGCAGAT TCAGCCGGGG 180
 CAGCAATATC CCGGCATGTT GCGCAGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG 240
 TCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG 300
 35 CCGTCGGATC CCGGCAGTTA TCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG 360
 ACGTTGCCGT CGCTATCCAT AGCACCGAGC GCGCGTCCGC AGACAGGGAA CGGACGCGCC 420
 CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT 480

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CACCGTCGGC GTCACCTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATCGGGGTG 540
 GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600
 AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660
 TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCACGCG TGGATAAACT 720
 5 GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGGCT 780
 GCGCGAGGGG CTGGGCGCCA GCTCGAAGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840
 TTTCGSCAAT GCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGCGCGCGA 900
 TCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960
 CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC 1020
 10 CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG 1080
 CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140
 GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200
 GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260
 CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320
 15 TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380
 GACGACGAC AAATCCTGGG CTAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440
 CGCCAGCATG GACAAATTC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500
 TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560
 GGCTGTCTGTC GGCATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620
 20 ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680
 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740
 ACGCACATTT TCCCGTTTAT TCGCGTGGT ACGCGCCACA ATCGCGATGG CATCTCTCTC 1800
 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860
 CAGATGAGA CACGTCTGCG ATAAATCTGT GCCGTAACTG GTTCTATCC GCCCCTTAG 1920
 25 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCTGTG CGCGGCGCGG 1980
 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040
 AAAATAGGGC AGTTTTTGCG TGGTATCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100
 GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CTTGATCGGT T 2141

30 The hypersensitive response elicitor from *Erwinia chrysanthemi* has 2
 hypersensitive response eliciting domains. The first domain extends, within SEQ.

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ID. No. 1, from amino acid 69 to amino acid 122, particularly from amino acid 85 to amino acid 116. The acidic unit in the first domain extends, within SEQ. ID. No. 1, from amino acid 69 to amino acid 102, particularly from amino acid 85 to amino acid 102. The alpha-helix in the first domain extends, within SEQ. ID. No. 1, from amino acid 102 to amino acid 122, particularly from amino acid 102 to amino acid 116. The second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 299, particularly from amino acid 256 to amino acid 292. The acidic unit in the second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 279, particularly from amino acid 261 to amino acid 279. The alpha-helix in the second domain extends, within SEQ. ID. No. 1, from amino acid 279 to amino acid 299, particularly from amino acid 279 to amino acid 292.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.

No. 3 as follows:

	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser	
	1				5					10					15		
20	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln	
				20					25					30			
	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn	
				35				40					45				
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met	
				50			55					60					
25	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu	
	65					70					75					80	
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu	
				85						90				95			
	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr	
30				100					105					110			
	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro	
				115				120					125				
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser	
				130			135					140					
35	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln	
	145					150					155				160		

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Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190
 5 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
 195 200 205
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
 210 215 220
 10 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
 225 230 235 240
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
 245 250 255
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
 260 265 270
 15 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
 275 280 285
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
 290 295 300
 20 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
 305 310 315 320
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
 325 330 335
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
 340 345 350
 25 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
 355 360 365
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380
 30 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400
 Gly Ala Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of
 about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10
 35 minutes. This hypersensitive response elicitor polypeptide or protein has substantially
 no cysteine. The hypersensitive response elicitor polypeptide or protein derived from
Erwinia amylovora is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zurnoff,

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D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence

5 corresponding to SEQ. ID. No. 4 as follows:

	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTCT	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
10	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAA ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGCGGTGG TGGGCTGATG	300
	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTGGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
15	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTGAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
20	GGTTCGTGCG TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGCTGGA CTACCAGCAG	840
	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTGAGC GCTGAATGAT	900
	ATCGGTACGC ACAGGCACAG TTCAACCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCGATAC	1020
	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC	1080
25	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
	ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
	CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

30 The hypersensitive response elicitor from *Erwinia amylovora* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 74, particularly from amino acid 45 to amino

acid 68. The acidic unit in the first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 57, particularly from amino acid 45 to amino acid 57. The alpha-helix in the first domain extends, within SEQ. ID. No. 3, from amino acid 57 to amino acid 74, particularly from amino acid 57 to amino acid 68. The second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 180, particularly from amino acid 145 to amino acid 170. The acidic unit in the second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 157, particularly from amino acid 145 to amino acid 157. The alpha-helix in the second domain extends, within SEQ. ID. No. 3, from amino acid 157 to amino acid 180, particularly from amino acid 157 to amino acid 170.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

15	ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTGCG CGGGTCTGTT CCAGTCCGGG	60
	GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT	120
20	CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTAAAGTC ACTGCTATCG	180
	CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT	240
	AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT	300
25	CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC	360
	CAGGGCGGCG GGCAGATCGG CGATAATCCT TTAAGTAAAG CCATGCTGAA GCTTATTGCA	420
30	CGCATGATGG ACGGCCAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC	480
	TCTTCGGTA CTTCTTCATC TGGCGGTTCC CCTTTAAGC ATCTATCAGG GGGGAAGGCC	540
	CCTTCGGCA ACTCCCTTC CGGCAACTAC TCTCCGTC GTACCTTCTC ACCCCATCC	600
35	ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCTTCTT CTCCCACCAA AGCAGCCGGG	660
	GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGCGAT CGGGGCCGGA	720
40	AATTCGGTGG CCTTCACCG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC	780
	GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA	840
	GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTITA TACTGGAAGA CGGTGCCAGC	900
45	CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC	960
	AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC	1020
50	AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACGTT CCTTCGAGCA CGCCTCTGAC	1080

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AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140
 TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200
 5 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260
 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320
 10 GCCAACCTGA AGGTGGCTGA ATGA 1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 6 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
 1 5 10 15
 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
 20 20 25 30
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
 35 40 45
 25 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
 50 55 60
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
 65 70 75 80
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
 85 90 95
 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
 35 100 105 110
 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp
 115 120 125
 40 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
 130 135 140
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
 145 150 155 160
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
 165 170 175
 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
 50 180 185 190
 Val Ser Thr Phe Ser Pro Pro S r Thr Pro Thr Ser Pro Thr Ser Pro
 195 200 205
 55 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
 210 215 220

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Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
 225 230 235 240
 5 Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
 245 250 255
 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
 260 265 270
 10 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
 275 280 285
 Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
 290 295 300
 15 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
 305 310 315 320
 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
 325 330 335
 20 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
 340 345 350
 Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
 355 360 365
 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
 370 375 380
 30 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
 385 390 395 400
 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
 405 410 415
 35 Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
 420 425 430
 40 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
 435 440 445

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It
 45 is also heat stable, protease sensitive, and suppressed by inhibitors of plant
 metabolism. The protein or polypeptide of the present invention has a predicted
 molecular size of ca. 4.5 kDa.

This hypersensitive response elicitor from *Erwinia amylovora* has 2
 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID.
 50 No. 6, from amino acid 5 to amino acid 64, particularly from amino acid 31 to amino
 acid 57. The acidic unit in the first domain extends, within SEQ. ID. No. 6, from
 amino acid 5 to amino acid 45, particularly from amino acid 31 to amino acid 45. The

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alpha-helix in the first domain extends, within SEQ. ID. No. 6, from amino acid 45 to amino acid 64, particularly from amino acid 45 to amino acid 64. The second domain extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 146, particularly from amino acid 116 to amino acid 140. The acidic unit in the second domain
 5 extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 131, particularly from amino acid 116 to amino acid 131. The alpha-helix in the second domain extends, within SEQ. ID. No. 6, from amino acid 131 to amino acid 146, particularly from amino acid 131 to amino acid 140.

Another potentially suitable hypersensitive response elicitor from
 10 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

15	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC	60
	CCTGTGGGGC ATGGTGTTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
	GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
20	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
	GGCTGTTTGG GGACGAAAAA ATTTTCAGG TCGGCACGCG AGGGCCAGCC AGGTACCACC	300
	CACAGCAAA GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
25	GAGGCGGGCG CGCCAGATGC GCGCGGTTTG ACCCGTTGCG GCGGCGTCAA ACGCGCAAT	420
	ATGGACGACA TGGCGGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
30	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
	AAAATGGCTC ACCCGGCTTC AGCCAAACGCC GCGGATCGCC TGCAGCATTC ACCGCGCAC	600
	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
35	ACGGCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAATCCAC CGCAGCCGCC CAACTGGGC	780
40	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC	840
	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
	GGAGCCGGGG TAACGCGCT GCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
45	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
50	CACCTGTTTG ATATCAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200

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	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCRA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTGGC AGCATCCTGC TGGCGCAGCG	1320
5	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAA TTCATATCCT GCATCCGGAG	1380
	CTGGGCGTAT GGCAATCTGC GGATAAGAT ACCCAGGCC AGCTGTCTCG CCAGGCAGAC	1440
	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCGA TAATAAATCC	1500
10	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
15	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTGCCGATG CCCACCAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTGGCC	1740
	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
20	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGTCATGA CCACCAGATT	1860
	TCTGGATTTT TCCATGACGA CCACGCCAG CTTAATGCGC TGGTGA AAAA TAACTTCAGG	1920
25	CAGCAGCATG CCTGCCCGTT GGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980
	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
	ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
30	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCCTGAA TATTAATCAG	2220
35	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT	2280
	AAACCGGAGC CGGGAGATGC CTTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
	GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC	2400
40	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGCGAC AAATCTCTAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTCTATGC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
45	CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
	CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
50	GGCTGGCAGC CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760
	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
55	TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
	AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACRAAT GTCCACGCCG	2940
	CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
60	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACTTCGT	3060
	CATAACGCGC CACAGCCAGA TTTGCAGAGC AAAGTGAAA CTCTGGATT AGGCGAACAT	3120
65	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGAGC AACTGGAGCA GAGTGCAACC	3180

	CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
	AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
5	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
	AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
10	AAGGGCGAGA TCCCCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCATA	3480
	TCGCGTTTAA TTTAGATAC CGTGACCATC GGTGAAGTGC ATGAAGTGC CGATAAGGCG	3540
	AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
15	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCAACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC	3720
20	TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCAGAGGGC	3780
	AGTGCCGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTGTG CCCTGGACAG TGGTGAAAGT	3840
	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCCTAGC	3900
25	AAGAAGGTGC CAGTTCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCCGCA CGGCGGGGTG	4020
30	AGTGGTAACA TCATGGTGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
	ACCAAGTGCAG GTAACGCCAG TGAAGGTTG AGCGCAAAAC ATAAATCAG CCCGGACTTG	4140
	CGTATCGGCG CTGCTGTGAG TGGCACCCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
35	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
40	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
	GGCAGTAAC CAAATGGTGT CACTGCCCCG GTTCTGCGG GGCTAAGTGC ATCGGCAAAC	4440
	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
45	GCCAGCAATA ACCGCCAAC CTTCCTCAAC GGGGTGCGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTGCGGAT CTCCCGGCA	4620
50	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
	ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
55	TTAGATGACG CTAAGCCCGC TGAACAACG CATATTTTAC AGCAGCATT CAGTGCAAAA	4860
	GATGTCGTG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT	4920
60	CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC	4980
	AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTGAGC TGCTACACAA ACATTTGAT	5040
65	GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100

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CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTGATG 5160
 GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220
 5 CGTGAAGAAG TGGGAGTACT TTCCAGGAT CGTAACAAC TCGGTGTAA ATCGGTCAGC 5280
 GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340
 AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400
 10 CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460
 CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

15

This DNA molecule is known as the *dspE* gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 8 as follows:

20

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
 1 5 10 15

25

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
 20 25 30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
 35 40 45

30

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80

35

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
 85 90 95

40

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
 115 120 125

45

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
 145 150 155 160

50

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
 165 170 175

55

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
 180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
 195 200 205

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Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
 210 215 220
 5 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
 225 230 235 240
 Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
 245 250 255
 10 Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
 260 265 270
 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
 275 280 285
 15 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
 290 295 300
 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
 305 310 315 320
 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
 325 330 335
 25 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
 340 345 350
 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
 355 360 365
 30 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
 370 375 380
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
 385 390 395 400
 35 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
 405 410 415
 40 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
 420 425 430
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
 435 440 445
 45 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
 450 455 460
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
 465 470 475 480
 50 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
 485 490 495
 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
 500 505 510
 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
 515 520 525
 60 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
 530 535 540
 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
 545 550 555 560
 65

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Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
 565 570 575
 5 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
 580 585 590
 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
 595 600 605
 10 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
 610 615 620
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
 625 630 635 640
 15 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
 645 650 655
 20 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
 660 665 670
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
 675 680 685
 25 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
 690 695 700
 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
 705 710 715 720
 30 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
 725 730 735
 35 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
 740 745 750
 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
 755 760 765
 40 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
 770 775 780
 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
 785 790 795 800
 45 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
 805 810 815
 50 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
 820 825 830
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
 835 840 845
 55 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
 850 855 860
 60 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
 865 870 875 880
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
 885 890 895

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Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
 900 905 910
 5 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
 915 920 925
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
 930 935 940
 10 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
 945 950 955 960
 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
 965 970 975
 15 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
 980 985 990
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
 995 1000 1005
 20 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
 1010 1015 1020
 25 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
 1025 1030 1035 1040
 Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
 1045 1050 1055
 30 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
 1060 1065 1070
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085
 35 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
 1090 1095 1100
 40 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
 1105 1110 1115 1120
 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135
 45 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150
 50 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165
 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180
 55 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
 1185 1190 1195 1200
 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
 1205 1210 1215
 60 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
 1220 1225 1230
 65 Ala Val Lys Ala Ph Ile Asn Ala Phe Lys Lys Glu His His Gly Val
 1235 1240 1245

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Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
 1250 1255 1260
 5 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
 1265 1270 1275 1280
 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
 1285 1290 1295
 10 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
 1300 1305 1310
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
 1315 1320 1325
 15 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
 1330 1335 1340
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
 1345 1350 1355 1360
 20 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
 1365 1370 1375
 25 Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
 1380 1385 1390
 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
 1395 1400 1405
 30 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
 1410 1415 1420
 35 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
 1425 1430 1435 1440
 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
 1445 1450 1455
 40 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
 1460 1465 1470
 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
 1475 1480 1485
 45 Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
 1490 1495 1500
 50 Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
 1505 1510 1515 1520
 Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
 1525 1530 1535
 55 Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
 1540 1545 1550
 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
 1555 1560 1565
 60 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
 1570 1575 1580

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His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
 1585 1590 1595 1600
 5 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
 1605 1610 1615
 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
 1620 1625 1630
 10 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
 1635 1640 1645
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
 1650 1655 1660
 15 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
 1665 1670 1675 1680
 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
 1685 1690 1695
 20 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
 1700 1705 1710
 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
 1715 1720 1725
 25 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
 1730 1735 1740
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
 1745 1750 1755 1760
 30 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
 1765 1770 1775
 35 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
 1780 1785 1790
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
 1795 1800 1805
 40 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
 1810 1815 1820
 45 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
 1825 1830 1835

50 This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

55 ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGCTGTAAA 60
 ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120
 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180
 60 GCTGACCCAC AAACCTCAAT AACCCGTGAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240

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GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAAGTGC ACAACGTGCG TTTATGTTT 300
 CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTATGCG ATATCGTTAG CGGCTTCATC 360
 5 GAACATGCGG CAGAAGTGGG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
 1 5 10 15
 15 Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
 20 25 30
 Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
 35 40 45
 20 Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
 50 55 60
 25 Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80
 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95
 30 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110
 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125
 35 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
 130 135

40 This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1 5 10 15
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
 20 25 30
 50 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60

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Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
 65 70 75 80
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85 90 95
 5 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
 100 105 110
 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125
 10 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
 130 135 140
 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
 145 150 155 160
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
 165 170 175
 15 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
 180 185 190
 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
 195 200 205
 20 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
 210 215 220
 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
 225 230 235 240
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
 245 250 255
 25 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
 260 265 270
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
 275 280 285
 30 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
 290 295 300
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335
 35 Asn Gln Ala Ala Ala
 340

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer,

- 5 "Pseudomonas syringae pv. syringae Harpin_{PS}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

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10 ATGCAGAGTC TCACTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCG      60
   GTACGTCTCG AAGCCGAGAC GACTGGCAGT ACGTGAGCA AGGCGCTTCA GGAAGTTGTC      120
   GTGAAGCTGG COGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA      180
   AAAGTGTGG CCAAGTCGAT GGCCGAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC      240
15 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG      300
   GACAGCGSCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC      360
   AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCGAAGAC      420
   GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC      480
   AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC      540
20 GAAACGGCTG CGTTCCGTTT GGCACCTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG      600
   AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCGAG CAGTTTTTCC      660
   AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC      720
   GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA      780
   TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCGCGAGAC CGGTACGTG      840
25 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG      900
   GGCCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT      960
   GCGCAAATCG CCACCTTGCT GGTCACTACG CTGCTGCAAG GCACCGCAA TCAGGCTGCA      1020
   GCCTGA

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30

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,

which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

5	TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG	60
	CTGAGTGC GC AGATTTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAGG	120
	CCTCTGAGTG CGGTGCGGAG CAATACCACT CTTCCTGCTG GCGTGTGCAC ACTGAGTGC	180
10	AGGCATAGGC ATTTCACTTC CTTCGTTGG TTGGGCATAT AAAAAAGGA ACTTTTAAAA	240
	ACAGTGCAAT GAGATGCCG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTC	300
15	AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC	360
	TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCAGCAT	420
	GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACACG CCACTCGATT TTTGCGGCT	480
20	AAGCGGCAAG AGTCCTCAAC CAAACAGTT CCGCGAGCAG AACACTCAGC AAGCGATCGA	540
	CCCGAGTGCA CTGTTGTTG GCAGCGACAC ACAGAAAGAC GTCAACTTC GCACGCCGGA	600
	CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATGCG	660
25	TAAATTGATC AGTGCAATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA	720
	GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT	780
30	CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG	840
	CGGTGATACG CCAAGCGCAA CAGGCGGTGG CCGCGGTGAT ACTCCGACCG CAACAGGCGG	900
	TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC	960
35	CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA	1020
	CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC	1080
40	CGGCAAGATC AATGTGTTGA AAGACACCAT CAAGTCCGC GCTGGCGAAG TCTTTGACGG	1140
	CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA	1200
	GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA	1260
45	CGAGGTCGAT GGCATCCAG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT	1320
	GCATGCCACG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT	1380
50	CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT	1440
	CAACGCCAAC ACTCACTTGA AATCGACAA CTTCAGGCC GACGATTTG GCACGATGGT	1500
	TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC	1560
55	TAACCACGGC AAGTTCGCCC TGGTGAAGAG CGACAGTGAC GATCTGAAGC TGGCAACGGG	1620
	CAACATGCGC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA	1680
60	CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAGGG GGTGGACTC	1729

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This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

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5      Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
      1              5              10              15

10     Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
      20              25              30

      Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
      35              40              45

15     Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
      50              55              60

      Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
      65              70              75              80

20     Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
      85              90              95

      Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
      100             105             110

25     Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
      115             120             125

30     Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
      130             135             140

      Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
      145             150             155             160

35     Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
      165             170             175

      Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
      180             185             190

40     Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
      195             200             205

      Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
      210             215             220

45     Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
      225             230             235             240

50     Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
      245             250             255

      Gln Gly Glu Asn Gln Lys Pro Met Ph Glu Leu Ala Glu Gly Ala Thr
      260             265             270

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Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
 275 280 285
 5 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
 290 295 300
 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
 305 310 315 320
 10 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
 325 330 335
 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
 340 345 350
 15 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
 355 360 365
 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
 370 375 380
 20 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
 385 390 395 400
 25 Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
 405 410 415
 Ala Ser Thr Gln His Thr Glu Leu
 420
 30

This protein or polypeptide is about 42.9 kDa.

This hypersensitive response elicitor from *Pseudomonas syringae* has 1
 35 hypersensitive response eliciting domain. This domain extends, within SEQ. ID. No.
 14, from amino acid 45 to amino acid 102, particularly from amino acid 58 to amino
 acid 92. The acidic unit in the first domain extends, within SEQ. ID. No. 14, from
 amino acid 45 to amino acid 79, particularly from amino acid 58 to amino acid 79.
 The alpha-helix in the first domain extends, within SEQ. ID. No. 14, from amino acid
 40 79 to amino acid 102, particularly from amino acid 79 to amino acid 92.

The hypersensitive response elicitor polypeptide or protein derived
 from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.
 ID. No. 15 as follows:

45 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln S r
 20 25 30

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Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45
 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
 5 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
 65 70 75 80
 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
 85 90 95
 10 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
 100 105 110
 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140
 15 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 20 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 25 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255
 30 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
 260 265 270
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300
 35 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335

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Gln Ser Thr Ser Thr Gln Pro Met
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.
ID. No. 16 as follows:

5	ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
	AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
	GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180
	GGCAACACCG GTAACACCGG CAACGCGCGG GCGAAGGAAG GCAATGCCAA CGCGGGCGGC	240
	AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
10	GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
	GACCTGGTGA AGCTGCTGAA GCGCGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG	420
	GGCAACGGCG TGGGCGGTGC CAACGCGGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480
	GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC	540
	GGCGGCGCGG GTGGCGGTGT CGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT	600
15	GGCGCAGGCG GTGCGAACCG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC	660
	GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACCG CAGCGAAGAC	720
	CAGGGCGGCC TCACCGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG	780
	ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC	840
	GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT	900
20	GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC	960
	GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG	1020
	ACGCAGCCGA TGTA	1035

- 25 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994),
- 30 which is hereby incorporated by reference.

The hypersensitive response elicitor from *Pseudomonas solanacearum* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID.

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No. 15, from amino acid 85 to amino acid 131, particularly from amino acid 95 to amino acid 123. The acidic unit in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 123. The alpha-helix in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 111. The second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 264, particularly from amino acid 229 to amino acid 258. The acidic unit in the second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 246, particularly from amino acid 229 to amino acid 264. The alpha-helix in the second domain extends, within SEQ. ID. No. 15, from amino acid 246 to amino acid 264, particularly from amino acid 246 to amino acid 258.

The N-terminus of the hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

15 Met Asp Gly Ile Gly Asn His Phe Ser Asn
1 5 10

20 The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

25 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15

Leu Leu Ala Met
20

30 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," *MPMI* 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not

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Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

- 5 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,"
- 10 Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992),
- 15 Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby
- 20 incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

- 25 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

- 30 Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

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Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids f

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SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µg/ml *E. coli* DNA. Suitable stringency conditions also include hybridization in a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C where hybridized nucleic acids remain bound when subject to washing the SSC buffer at a temperature of 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of 42°C where hybridized nucleic acids remain bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

A particularly advantageous aspect of the present invention involves utilizing a protein having a pair or more, particularly 3 or more, coupled domains. These domains can be from different source organisms. When a DNA molecule encoding such a protein is prepared, it can be advantageously used to make transgenic plants. The use of a gene encoding such domains, as opposed to a gene encoding a full length hypersensitive response elicitor, has a number of benefits. Firstly, such a gene is easier to synthesize. More significantly, the use of a plurality of domains together from different source organisms can impart their combined benefits to a transgenic plant.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant

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DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria

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transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;
microorganisms such as yeast containing yeast vectors; mammalian cell systems
infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected
with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression
5 elements of these vectors vary in their strength and specificities. Depending upon the
host-vector system utilized, any one of a number of suitable transcription and
translation elements can be used.

Different genetic signals and processing events control many levels of
gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

10 Transcription of DNA is dependent upon the presence of a promoter
which is a DNA sequence that directs the binding of RNA polymerase and thereby
promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from
those of procaryotic promoters. Furthermore, eucaryotic promoters and
accompanying genetic signals may not be recognized in or may not function in a
15 procaryotic system, and, further, procaryotic promoters are not recognized and do not
function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon
the presence of the proper procaryotic signals which differ from those of eucaryotes.
Efficient translation of mRNA in procaryotes requires a ribosome binding site called
20 the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short
nucleotide sequence of mRNA that is located before the start codon, usually AUG,
which encodes the amino-terminal methionine of the protein. The SD sequences are
complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably
promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct
25 positioning of the ribosome. For a review on maximizing gene expression, see
Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby
incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote
transcription). For the purposes of expressing a cloned gene, it is desirable to use
30 strong promoters in order to obtain a high level of transcription and, hence,
expression of the gene. Depending upon the host cell system utilized, any one of a
number of suitable promoters may be used. For instance, when cloning in *E. coli*, its

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bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, plant cells as well as

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prokaryotic and eukaryotic cells, such as bacteria, virus, yeast, mammalian, insect cells, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance to plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart stress resistance.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance to the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash,

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pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting
10 disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

 The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of
15 pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas*
20 *campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

 With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant
25 growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit
30 and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their

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growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any
5 form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants,
10 preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on
15 over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm),
20 pepper maggot, and tomato pinworm. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

Another aspect of the present invention is directed to imparting stress resistance to plants. Stress encompasses any environmental factor having an adverse
25 effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy
30 metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

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The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.

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Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g.,

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dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies.

- 5 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

- The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are
10 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

- Another method of introducing the DNA molecule into plant cells is to
15 infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration
20 medium without antibiotics at 25-28°C.

- Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized
25 only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

- Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A.*
30 *rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

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After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, control of insects on the plant, and/or stress resistance. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance. While not

wishing to be bound by theory, such disease resistance, growth enhancement, insect control, and/or stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

15

EXAMPLES

Example 1 - Bacterial Strains and Plasmids

Escherichia coli DH5 and BL21 were purchased from Gibco BRL (Rockville, MD) and Novagen (Madison, WI) respectively.

pET28 plasmids were from Novagen (Madison, WI).

All restriction enzymes (e.g., NdeI and HindIII), T4 DNA ligase, Calf intestinal alkaline phosphatase (CIP), and PCR reagents were from Gibco BRL (Rockville, MD).

Oligonucleotides were synthesized by Lofstrand Labs Ltd (Gaithersburg, MD).

Chemically synthesized polypeptides were synthesized by Bio-Synthesis (Lewisville, TX).

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Example 2 - Construction of Truncated Gene Encoding Harpin

Fragments of genes encoding harpin proteins were constructed in pET28 vector and expressed in *E. coli* as follows;

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1. HrpN fragments were PCR amplified from the pCPP2139 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
2. HrpZ fragments were PCR amplified from the pSYH10 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
3. PopA fragments were PCR amplified from the pBS::popA plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
4. HrpW fragments were PCR amplified from the pCPP1233 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.

All truncated fragments were amplified by PCR with full length harpin DNA as the template.

- 15 Oligonucleotides corresponding to the truncated N-terminal sequence were started /modified with a Nde I site (which serves as an initiation codon of methionine (ATG)). Oligonucleotides corresponding to a C-terminal sequence contained a UAA stop codon followed by a Hind III site.

PCR was carried in a 0.5 ml tube with GeneAmp™ 9600 and 9700 (PE Applied Biosystems, Branchburg, New Jersey). 45 µl of SuperMix™ (Gibco BRL, Rockville, MD) was mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and diH₂O to fill the final volume to 50 µl. After heating the mixture at 95°C for 2 min., PCR was performed for 30 cycles at 94°C for 1 min., 58°C for 1 min. and 72°C for 1.5 min. Amplified DNAs were purified with QIAquick PCR purification kit (QIAGEN Inc., Vencia, CA), digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:24:1), and precipitated with ethanol. 5 µg of pET28(b) vector DNA was digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with calf intestinal alkaline phosphatase treatment for 30 min. at 37°C to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried at 14°C for 12 hours in a 15 µl mixture containing about 50 to

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100 ng of digested pET28(b), 10 to 30 ng of targeted PCR fragments, and 1 unit of T4 DNA ligase. 5 µl of ligation solution was added to 100 µl of DH5α/XL1-Blue competent cells, placed in 15 ml Falcon tube, and incubated on ice for 30 min. After heat shock at 42°C for 45 seconds, 0.9 ml SOC solution (20 g bacto-tryptone, 5 g bacto-yeast extracts, 0.5 g NaCl, 20 mM glucose in one liter) was added into the tube and incubated at 37°C for 1 hour. 20 µl of transformed cells were plated onto LB agar plate with 30 µg/ml of kanamycin and incubated at 37°C for 14 hours. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared in a 2 ml culture with QIAprep Miniprep kit according to the manufacture's instruction. The DNA sequence of truncated harpin constructions was verified with restriction enzyme analysis and sequencing analysis. Plasmids with the desired DNA sequence were transferred into the BL21 strain with a standard chemical transformation method as indicated above.

15 Example 3 - Expression of Proteins

A single clone of *E. coli* with a constructed gene was grown overnight at 37°C in LB with kanamycin. A proper amount of overnight culture was transferred to 50 to 500 ml LB and incubated at 37°C until OD600 reached 0.5 to 0.8. IPTG was added to the culture which was further incubated at room temperature for a period of 5 hour to overnight. Alternatively, a proper amount of overnight culture was transferred to 50 to 500 ml of ½ TB with lactose medium (6 g bacto-trypton, 12 g bacto-yeast extract, 75 g lactose in one liter). After incubation at 37°C until the OD600 reached 0.5 to 0.8, the culture was incubated at room temperature for a period of 5 hours to overnight.

All bacterial cells were harvested by centrifugation and resuspended in 1:5 TE buffer (10 mM Tris, pH 8.5 and 1 mM EDTA). The cells were disrupted by sonication and clarified by centrifugation. Supernatants were then infiltrated into tobacco leaves for HR testing.

Heat treatment (i.e. boiling for 1 to 10 min.) was used to achieve further purification.

All truncated fragments of genes encoding harpin protein were expressed in *E. coli*/ BL-21, DE3 strain with an N-terminal His-tag and 20 to 21

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amino acid residues generated from the expression vector sequence. The His-tag sequence did not affect the HR activity of the proteins. In some cases, Ni-Agarose beads were added into supernatant solution and mixed at 4°C to room temperature for a period of 30 min. to overnight. The proteins bound to the Ni-Agarose beads were washed by 0.1 M imidazole buffer, and proteins were eluted with 0.6 to 1.0 M imidazole. After dialysis against 10 mM Tris, pH 8.5 buffer, the proteins were infiltrated into tobacco leaves for HR testing.

For proteins expressed in *E. coli* that were difficult to dissolve in water, total cells were resuspended and sonicated in 8 M urea buffer (0.1M Na-phosphate, 10 mM Tris buffer, pH8.0). The total cell lysate was centrifuged, and supernatants were collected. Ni-agarose was added into the supernatants and mixed gently at room temperature for 30 min. The Ni-agarose resin was washed with buffer (8 M urea, 0.1 M Na-phosphate, 10 mM Tris buffer, pH6.3). The proteins were eluted with elution buffer (8 M urea, 0.1 M EDTA, 0.1 M Na-phosphate, 10 mM Tris buffer, pH 6.3) and dialyzed against buffer (pH 8.5, 10 mM Tris) with stepwise decreased urea. If the proteins still were insoluble in buffer, the solution pH was adjusted to 9 to 11 and sonicated at room temperature for 1 to 5 min.

Chemically synthesized polypeptides were dissolved in 10 mM Tris, pH 6.5 to 11 buffers depending on their solubility.

A hypersensitive response ("HR") assay was performed by infiltration of 0.1 to 0.3 ml of serial diluted protein solutions into tobacco leaves (cv. Xanth). All HR data shown in these examples were recorded from 48 hours after infiltration.

Example 4 - Quantification of Proteins

All expressed proteins were checked with pre-cast 4-20% SDS polyacrylamide gel electrophoresis (SDS-PAGE) from Novex (San Diego, CA). After electrophoresis, the gel was stained with Coomassie R-250 solution (0.1% Coomassie R-250, 10% Acetate Acid, 40% ethanol) for 1 to 4 hours and destained with destaining solution (8% acetate acid and 25% ethanol) overnight. The density of corresponding bands were compared to standard proteins, which were either purchased from Novex or were from quantitative standard harpin protein produced by Eden Bioscience (Bothell, Washington).

Example 5 - Classification of Harpin Proteins

Since harpin proteins share common biochemical and biophysical characteristics as well as biological functions, based on their unique properties, HR elicitors from various pathogenic bacteria should be viewed as belonging to a new protein family—i.e. the harpin protein family. The harpin protein can be classified into at least four subfamilies based on their primary structure and isolated sources. As set forth in Table 1, those subfamilies are identified by the designation N, W, Z, A, etc.

Table 1 - Subfamilies of Harpin Proteins

Harpin proteins	Isolated Source	Classified Subfamily	pI	Amino acids	Heat stable	Core structure
HrpN _{Pa}	<i>E. amylovora</i>	N	4.42	403	Yes	No
HrpN _{Gch}	<i>E. chrysanthemi</i>	N	6.51	340	Yes	No
HrpN _{Gcc}	<i>E. carotovora</i>	N	5.82	356	Yes	No
HrpN _{St}	<i>E. stewartii</i>	N	N/A	N/A	Yes	No
HrpW _{Pa}	<i>P. syringae</i>	W	4.43	424	Yes	No
HrpW _{Pa}	<i>E. amylovora</i>	W	4.46	447	Yes	No
HrpZ _{Pa}	<i>P. syringae</i>	Z	3.95	341	Yes	No
PopA1	<i>R. solanacearum</i>	A	4.16	344	Yes	No

Example 6 - Analysis of the Structural Units of an HR Domain

The sequence of amino acids that alone could elicit a hypersensitive response in plants (i.e. HR domains) has been investigated in different ways. It was reported that a carboxyl-terminal 148 amino acid portion of HrpZ_{Pa} is sufficient and necessary for HR (He et al., "Pseudomonas Syringae pv. Syringae Harpin_{Pa}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266.(1993), which is hereby incorporated by reference). With truncated HrpZ fragments, it was determined that an N-terminal 109 amino acids and C-terminal 216 amino acids of HrpZ_{Pa}, respectively, were found to elicit HR (Alfano et al., "Analysis of the Role of the Pseudomonas Syringae pv. Syringae HrpZ Harpin in Elicitation of the Hypersensitive Response in Tobacco Using

Functionally Non-polar hrpZ Deletion Mutations, Truncated HrpZ Fragments, and hrnA Mutations," Molecular Microbiology 19:715-728 (1996), which is hereby incorporated by reference). Jin et al., "A Truncated Fragment of Harpin_{ps} Induces Systemic Resistance to Xanthomonas campestris pv. Oryzae in Rice," Physiological and Molecular Plant Pathology 51:243-257 (1997), which is hereby incorporated by reference, reported that a truncated HrpZ_{ps} with an N-terminal of 137 amino acids elicited a hypersensitive response in tobacco and induced systemic acquired resistance (i.e. SAR) in rice. After digestion with protease, a hypersensitive response active fragment of HrpN_{Ea} was isolated and found to span amino acids 137 to 204 of HrpN_{Ea}. It was found that a 98 residue of N-terminal HrpN_{Ea} fragment was the smallest bacterially produced peptide that displayed HR-eliciting activity (Laby, "Molecular Studies on Interactions Between Erwinia Amylovora and its Host and Non-host Plants," Doctoral Thesis in Cornell University (1997), which is hereby incorporated by reference).

A series of HrpN_{Ea} fragments have been generated with His-tag fusion at the N-terminal of the polypeptides and a polypeptide (HrpN_{Ea}137180), located at position of 137 to 180 amino acid residue of HrpN_{Ea}, was identified to elicit HR activity in tobacco.

20 Example 7 - Analysis of Secondary Structure of HR Domains

The DNA and primary protein sequence of the HrpN_{Ea}137180 show no any homologues among other hypersensitive response elicitors.

Analyses of the secondary structure of the fragment of HrpN_{Ea}137180 revealed, with the aid of the computer program Clone Manger5 (Scientific & Educational Software, Durham, NC), that there was a beta-form, a beta-turn, and unordered forms. One typical α -helical segment of residues at 157-170 was found in the HrpN_{Ea}137180 polypeptide. To determine the function of this structure, polypeptides with a disrupted α -helical structure were generated and hypersensitive response results were evaluated. As shown in Table 2, a complete alpha-helix unit (H unit), probably with a length greater than 12 amino acid residues, is need for hypersensitive response activity.

Table 2 - Effect of Alpha-helix Structure

Fragment name	Amino acid	HR*	Structure	Source
HrpN _{Ea} 137180	137-180 (44) pI = 3.10	+ <5 µg/ml	Complete H	E.coli expressed peptide
HrpN _{Ea} 137166	137-166 (30) pI = 3.29	-	disrupted H	Synthesized peptide
HrpN _{Ea} 76168	76-168 pI = 3.39	-	disrupted H	E.coli expressed peptide

5 The α -helical unit plays an important role in hypersensitive response activity; however, it was found that an α -helix unit alone did not achieve HR (Table 3).

 Therefore, hypersensitive response eliciting domains contain more than one structure unit. Besides the core α -helical unit, there is an acidic unit that has no
10 typical secondary structure feature but is rich in acidic amino acids. This relaxed structure, having a sheet and random turn, is designated as an acidic unit (A unit).

 Although the acidic unit is important in achieving a hypersensitive response, it alone, like the α -helical unit alone, did not elicit a hypersensitive response.

15 A synthetic polypeptide, HrpN_{Ea}140176, that included both A and H structure, spanning amino acids 140 to 176 of HrpN_{Ea}, gave full activity of HR. Sequence analysis by major search engines revealed no global primary sequence similarity in the databases to HrpN_{Ea}140176, even among the harpin protein families.

20 **Table 3 - Effect of Acidic Unit on Hypersensitive Response (HR) Activity**

Fragment name	Amino acid	HR*	Structure (A or H)**	Source
HrpN _{Ea} 140176	140-176 (37) pI=3.17	+ <5 µg/ml	A + H	Synthesized peptide
HrpN _{Ea} 157170	157-170 (14) pI = 6.94	-	H	Synthesized peptide
HrpN _{Ea} 137156	137-156 (20) pI = 2.67	-	A	Synthesized peptide

Example 8 - Hypersensitive Response Domain Structure of HrpN_{Ea}

Four α -helical regions with at least 12 amino acid residues were found in HrpN_{Ea} based on computer analysis with the program Clone Manager 5 (Scientific & Educational Software, Durham, NC), which predicts the secondary structure of protein from the primary sequence by the method of Garnier-Osguthorpe-Robson.

It is believed that a hypersensitive response domain includes two structural units, the α -helix (H) and the acidic unit (A). Another hypersensitive response domain, spanning amino acids 43 to 70 in HrpN_{Ea}, was found. A minimal sequence of 12 to 14 AA residues of both the H and A units is believed to be needed. The chemically synthesized polypeptide of HrpN_{Ea}4370 gave full HR activity in tobacco. Thus, a second HR domain has been discovered based on purely secondary structure analysis and prediction.

To further test the hypothesis that the A and H units are needed to achieve a hypersensitive response, an approach of unit exchange (i.e. swapping an acidic unit from one HR domain to another HR domain) was designed. A polypeptide of HrpN_{Ea}Dswap, which consisted of the acidic unit of a hypersensitive response domain (HrpN_{Ea}140176), spanning amino acids 136 to 156 of HrpN_{Ea}, and the α -helical unit of another hypersensitive response domain (HrpN_{Ea}4370), spanning amino acids 57 to 70 of HrpN_{Ea}, was chemically synthesized. This polypeptide swapped two structural units of A and H between two hypersensitive response domains of HrpN_{Ea}4370 and HrpN_{Ea}140176. The HrpN_{Ea}Dswap gave a hypersensitive response activity in tobacco (Table 4). This result shows that the structural characteristic of an HR domain determines its activity, and structural analysis can be used to determine hypersensitive response activity.

Table 4 - Two Structural Units Determine Hypersensitive Response Activity

Fragment name	Amino acid	HR	Structure Type	Source
HrpN _{Ea} 4370	43-70 (28) pI= 3.09	+ <5 μ g/ml	A + H	Synthesized peptide Partial soluble
HrpN _{Ea} Dswap	HrpN136156 (A)+ HrpN5770 (H) pI=2.67	<20 μ g/ml	A unit from HrpN _{Ea} 140176 + H unit from HrpN _{Ea} 4370	Synthesized peptide Partial soluble

Example 9 - Prediction of Hypersensitive Response Domains Among Proteins in Harpin Family

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The secondary structure which indicates the presence of a hypersensitive response domain in HrpNEa was used to identify other harpin proteins, including proteins classified as different subfamilies. Structural prediction of a hypersensitive response domain among harpin proteins was carried according to

10 following criteria:

1. There are two structural units in a hypersensitive response domain, including:
 - a. A stable α -helix unit with 12 or more amino acids in length and
 - 15 b. An hydrophilic, acidic unit with 12 or more amino acids in length which could be a beta-form, a beta-turn, and unordered forms.
2. The pI of a hypersensitive response domain should be acidic and, in general, below 5.
- 20 3. The minimal size of an HR domain is from about 28 to 40 AA residues.

Putative HR domains have been identified to fit the criteria by computer analysis among harpin protein family (Table 5).

Table 5 - Predication of Hypersensitive Response Domains Among Harpin Proteins

HR domain	Isolated Source	Predicted region*	pI	Structure
HrpN _{Es} -1	<i>E. amylovora</i>	43-70	3.09	A + H
HrpN _{Es} -2	<i>E. amylovora</i>	140-176	3.17	A + H
HrpN _{EcH} -1	<i>E. chrysanthemi</i>	78-118	5.25	A + H
HrpN _{EcH} -2	<i>E. chrysanthemi</i>	256-295	4.62	A + H
HrpN _{EcC} -1	<i>E. carotovora</i>	25-63	4.06	A + H
HrpN _{EcC} -2	<i>E. carotovora</i>	101-140	3.00	A + H
HrpW _{Ps} -1	<i>P. syringae</i>	52-96	4.32	A + H
HrpW _{Es} -1	<i>E. amylovora</i>	10-59	4.53	A + H
HrpZ _{Ps} -1	<i>P. syringae</i>	97-132	3.68	A + H
HrpZ _{Ps} -2	<i>P. syringae</i>	153-189	3.67	A + H
HrpZ _{Ps} -3	<i>P. syringae</i>	271-308	3.95	A + H
PopA1 _R -1	<i>R. solanacearum</i>	92-125	3.75	A + H
PopA1 _R -2	<i>R. solanacearum</i>	206-260	3.62	A + H

5 *Amino acid residue position

Example 10 - Hypersensitive Response Activity of Select Synthesized Polypeptides

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Polypeptides were produced by expression in either *E. coli* or by chemical synthesis. Based on prediction of solubility and stability of a particular peptide, in some cases, a broader region of AA residues in addition to the essential units were also synthesized to increase solubility of the peptides. The identification of

15 HR domains among four subfamilies of harpin protein demonstrated this (Table 6).

Table 6 - Hypersensitive Response Activity of Select Synthesized Polypeptides

HR domain	Isolated Source	Synthesized region	pI	Source	HR activity
HrpN _{ES} -1	<i>E. amylovora</i>	43-70	3.09	Chemical Synthesized	+ < 5 µg/ml
HrpN _{ES} -2	<i>E. amylovora</i>	140-176	3.17	Chemical Synthesized	+ < 5 µg/ml
HrpW _{ES} -2	<i>E. amylovora</i>	10-59	4.53	E.coli expressed	+ < 5 µg/ml
HrpZ _{PS} -1	<i>P. syringae</i>	97-132	3.68	Chemical Synthesized	+ < 20 µg/ml
HrpZ _{PS} -1	<i>P. syringae</i>	153-189	3.69	E.coli expressed	+ < 5 µg/ml
PopA1 _{RS} -1	<i>R. solanacearum</i>	92-125	3.75	Chemical Synthesized	+ < 5 µg/ml
PopA1 _{RS} -2	<i>R. solanacearum</i>	206-260	3.62	E.coli expressed	+ < 5 µg/ml

5 **Example 11 - Construction of Hypersensitive Response Domains in a Protein Expression Cassette**

Polypeptides with a harpin protein hypersensitive response domain were expressed in *E. coli*. PCR was used to amplify desired areas of genes encoding harpin proteins and cloned into an expression vector, e.g. pET28a. A pair of PCR primers with unique flanking sequences were designed to create a universal expression cassette, as shown in Figure 1, for expression of a fragment of harpin protein. Each amplified DNA fragment has a protein translation start codon of ATG in a restriction enzyme Nde I site which might add an extra amino acid of methionine into a polypeptide. Each amplified DNA fragment has a protein translation stop codon of TAA. Each amplified fragment contained two restriction enzyme sites of EcoR V and Sma I, which gave 4 extra in-frame amino acids expressed as Pro-Gly at the N-terminal and Asp-Ile at the C-terminal, respectively. Those two sites are essential to allow two or more expression cassettes to be linked in a specific order and in frame with a minimum number of amino acids being introduced. Cassette A was first digested by EcoR V, ligated to cassette B, and digested with Sma I to produce a new expression cassette C which coupled the two fragments together with two extra amino acids (i.e. Asp-Gly), which are common amino acids in hypersensitive response domains. The newly formed cassette C still contained the same 5' and 3' flanking sequences as original cassettes A and B and maintained the ability to be

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coupled by another cassette. Bgl II and Bam HI sites in the cassette permit the cassette to be linked in frame into a concatomer with a correct orientation. The strategy is that digestion of DNA with Bgl II and Bam HI results in compatible ends that would be ligated with each other but could not be cut by either enzymes after ligation. For example, a DNA fragment encoding a hypersensitive response domain in a cassette could be digested by restriction enzymes of Bgl II and Bam HI separately, digested DNA fragments could be ligated in a ligation solution also including both Bgl II and Bam HI enzymes, any ligated ends with Bgl II or Bam HI sites could be digested by the enzymes, and only those ligated sites between Bgl II and Bam HI could remain.

Example 12 - Building Blocks for Creating Superharpins that have Higher Biological Efficacy

Hypersensitive response domains were identified and isolated from several harpin proteins. With the combination of those HR domains, new polypeptides (i.e. superharpins) that have higher HR potency and have enhanced ability to induce disease resistance, impart insect resistance, enhance growth, and achieve environmental stress tolerance. Superharpins could be one HR domain repeat units (concatomer), different combinations of HR domains, and/or biologically active domains from other elicitors. Part of the domains from different harpin proteins and other elicitors were constructed into the universal expression cassette as shown on Example 11 and designated as superharpin building blocks. Table 7 lists some superharpin building blocks which were expressed in pET-28a(+) vector with a His-tag sequence at their N-terminal.

Table 7 - Superharpin Building Blocks including pET-28a(+) his-tag Leader Sequence

Domain Sequence	Source	MW (kDa)	#a.a.	pI	Soluble	(Structurally) Heat Stable
A	PopA70-146	10.69	104	6.48	Yes	Yes
(N _N)	HrpNEa40-80	6.754	68	6.78	N/A	N/A
(N _N) ₂	Dimer of HrpNEa40-80	10.84	111	6.13	N/A	N/A
(N _N) ₃	Triplemer of HrpNEa40-80	14.93	154	5.63	N/A	N/A
(N _N) ₄	Tetramer of HrpNEa40-80	19.01	197	4.95	N/A	N/A
(N _C)	HrpNEa140-180	7.224	68	5.01	Yes	Yes
(N _C) ₂	Dimer of HrpNEa140-180	11.78	111	3.98	Yes	Yes
(N _C) ₃	Triplemer of HrpNEa140-180	16.34	154	3.72	Yes	Yes
(N _C) ₄	Tetramer of HrpNEa140-180	20.89	197	3.58	Yes	Yes
(N _C) ₁₀	Cancatomer (10 repeating units of HrpNEa140-180)	48.23	455	3.28	N/A	N/A
(N _C) ₁₆	Cancatomer (16 repeating units of HrpNEa140-180)	75.57	713	3.18	N/A	N/A
W	HrpWEa10-59	7.986	77	6.48	N/A	N/A
Z _N	HrpZ90-150	8.087	78	5.38	Yes	Yes
Z ₂₆₆₋₃₀₈	HrpZ266-308	7.029	70	6.40	Yes	Yes
his-tag leader seq.		2.045	19	11.04		

5

Example 13 - Superharpins with Stacked HR Domains and their Biological Activities

There are numerous polypeptides could be generated with different combinations of HR domains or by stacking HR domains and repeating units in order. Selective combination or stacking of HR domains isolated from harpin proteins or other elicitors can be designed to achieve a targeted disease resistance spectrum. See Table 8 for superharpins prepared by stacking of HR building blocks listed on Table 7. All three listed superharpins (i.e. SH-1, SH-2, SH-3) were constructed into a pET28(a) vector and expressed in *E. coli*. Recombinant proteins were partially purified and quantified by SDS-PAGE with purified Harpin N protein as a quantitative standard.

Table 8 - Properties of Superharpins

Protein	Domain Sequence	MW (kDa)	# a.a.	pI	Soluble	Heat Stable
SH-1	*W(N _N) ₄ A(N _C) ₄ Z ₂₆₆₋₃₀₈	54.955	545	3.69	Yes	Yes
SH-2	*W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈	52.341	519	3.54	Yes	Yes
SH-3	*W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈ A	60.375	598	3.67	Yes	Yes
HrpNEa	HrpN from <i>E. amylovora</i>	39.697	403	4.42	Yes	Yes

- 5 Bioassays for hypersensitive response on tobacco leaves (HR), percentage of TMV reduction on tobacco leaves, and plant growth enhancement with tomato showed that superharpins had higher (up to 2 to 10 fold greater) HR potency compared with HrpN from *E. amylovora*. This also demonstrated that superharpins have better performance on % TMV reduction and plant growth enhancement assay.
- 10 See Table 9.

Table 9 - Biological Activities of Superharpins

Protein	Domain Sequence	Elicit HR (~µg/ml)	% TMV reduction on tobacco		% Plant Growth Enhancement	
			10 µg/ml	1 µg/ml	10 µg/ml	1 µg/ml
SH-1	W(N _N) ₄ A(N _C) ₄ Z ₂₆₆₋₃₀₈	0.66	83	79	7.49	9.83
SH-2	W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈	0.13	84	60	11.05	7.30
SH-3	W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈ A	0.15	77	55	11.07	10.00
HrpNEa	HrpN from <i>E. amylovora</i>	1-3	55	10	11.68	N/A

15

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated hypersensitive response elicitor protein comprising
an isolated pair or more of spaced apart domains, each comprising an acidic portion
5 linked to an alpha-helix and capable of eliciting a hypersensitive response in plants.
2. A protein according to claim 1, wherein the protein is
recombinant.
- 10 3. An isolated nucleic acid molecule encoding a protein according
to claim 1.
4. A nucleic acid molecule according to claim 3, wherein each
domain is from a different source organism.
- 15 5. A nucleic acid molecule according to claim 3, wherein there are
3 or more spaced apart domains.
6. An expression vector containing a nucleic acid molecule
20 according to claim 3 which is heterologous to the expression vector.
7. An expression vector according to claim 6, wherein the nucleic
acid molecule is positioned in the expression vector in sense orientation and correct
reading frame.
- 25 8. A host cell transformed with the nucleic acid molecule
according to claim 3.
9. A host cell transformed according to claim 8, wherein the host
30 cell is selected from the group consisting of a plant cell, a eukaryotic cell, and a
procaryotic cell.

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10. A host cell according to claim 8, wherein the nucleic acid molecule is transformed with an expression system.

11. A transgenic plant transformed with the nucleic acid molecule
5 of claim 3.

12. A transgenic plant according to claim 11, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive,
10 cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

13. A transgenic plant according to claim 11, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.
15

14. A transgenic plant according to claim 11, wherein the plant is a
20 monocot.

15. A transgenic plant according to claim 11, wherein the plant is a dicot.

16. A transgenic plant according to claim 11, wherein each domain is from a different source organism.
25

17. A transgenic plant according to claim 11, wherein there are 3 or more spaced apart domains.
30

18. A transgenic plant seed transformed with the nucleic acid molecule of claim 3.

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19. A transgenic plant seed according to claim 18, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.
20. A transgenic plant seed according to claim 18, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.
21. A transgenic plant seed according to claim 18, wherein the plant is a monocot.
22. A transgenic plant seed according to claim 18, wherein the plant is a dicot.
23. A method of imparting disease resistance to plants comprising: applying a protein according to claim 1 to a plant or a plant seed under conditions effective to impart disease resistance to the plant or to a plant grown from the plant seed.
24. A method according to claim 23, wherein the protein is applied to a plant.
25. A method according to claim 23, wherein the protein is applied to a plant seed and further comprising: planting the plant seed under conditions effective to impart disease resistance to a plant grown from the plant seeds.

26. A method of enhancing plant growth comprising:
applying a protein according to claim 1 to a plant or a plant seed under
conditions effective to enhance growth of the plants or of a plant grown from the plant
seed.
- 5 27. A method according to claim 26, wherein the protein is applied
to a plant.
28. A method according to claim 26, wherein the protein is applied
10 to a plant seed and further comprising:
planting the plant seeds under conditions effective to enhance growth
of a plant grown from the plant seed.
29. A method of controlling insects comprising:
15 applying a protein according to claim 1 to a plant or a plant seed under
conditions effective to control insects.
30. A method according to claim 29, wherein the protein is applied
to a plant.
- 20 31. A method according to claim 29, wherein the protein is applied
to a plant seed and further comprising:
planting the plant seed under conditions effective to grow a plant from
the plant seed and to control insects.
- 25 32. A method of imparting stress resistance to plants comprising:
applying a protein according to claim 1 to a plant or a plant seed under
conditions effective to impart stress resistance to the plant or to a plant grown from
the plant seed.
- 30 33. A method according to claim 32, wherein the protein is applied
to a plant.

34. A method according to claim 32, wherein the protein is applied
to a plant seed and further comprising:
planting the plant seed under conditions effective to impart stress
5 resistance to a plant grown from the plant seed.
35. A method of imparting disease resistance to plants comprising:
providing a transgenic plant or transgenic plant seed containing the
nucleic acid according to claim 3 and
10 planting the transgenic plant or transgenic plant seed under conditions
effective to impart disease resistance to the plant or to a plant grown from the plant
seed.
36. A method according to claim 35, wherein a transgenic plant is
15 provided.
37. A method according to claim 35, wherein a transgenic plant
seed is provided.
38. A method of enhancing growth of plants comprising:
providing a transgenic plant or transgenic plant seed containing the
nucleic acid according to claim 3 and
20 planting the transgenic plant or transgenic plant seed under conditions
effective to enhance growth of the plant or of a plant grown from the plant seed.
39. A method according to claim 38, wherein a transgenic plant is
25 provided.
40. A method according to claim 38, wherein a transgenic plant
30 seed is provided.
41. A method of controlling insects comprising:

providing a transgenic plant or transgenic plant seed containing the nucleic acid according to claim 3 and

planting the transgenic plant or transgenic plant seed under conditions effective to control insects on the plant or on a plant grown from the plant seed.

5

42. A method according to claim 41, wherein a transgenic plant is provided.

43. A method according to claim 41, wherein a transgenic plant seed is provided.

10

44. A method of imparting stress resistance to plants comprising: providing a transgenic plant or transgenic plant seed containing the nucleic acid according to claim 3 and

15

planting the transgenic plant or transgenic plant seed under conditions effective to impart stress resistance to the plant or to a plant grown from the plant seed.

20

45. A method according to claim 44, wherein a transgenic plant is provided.

46. A method according to claim 44, wherein a transgenic plant seed is provided.

25

47. An isolated hypersensitive response elicitor protein comprising, in isolation, a domain comprising an acid portion linked to an alpha-helix and capable of eliciting a hypersensitive response in plants.

30

48. A protein according to claim 47, wherein the protein is recombinant.

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49. An isolated nucleic acid molecule encoding a protein according to claim 47.

50. An isolated nucleic acid molecule according to claim 49,
5 wherein there are at least 2 domains, each from a different source organism.

51. An isolated nucleic acid molecule according to claim 49,
wherein there are 3 or more coupled domains.

52. An expression vector containing a nucleic acid molecule
10 according to claim 49 which is heterologous to the expression vector.

53. An expression vector according to claim 52, wherein the
nucleic acid molecule is positioned in the expression vector in sense orientation and
15 correct reading frame.

54. A host cell transformed with the nucleic acid molecule
according to claim 49.

55. A host cell transformed according to claim 54, wherein the host
20 cell is selected from the group consisting of a plant cell, a eukaryotic cell, and a
prokaryotic cell.

56. A host cell according to claim 54, wherein the nucleic acid
25 molecule is transformed with an expression system.

57. A transgenic plant transformed with the nucleic acid molecule
of claim 49.

58. A transgenic plant according to claim 57, wherein the plant is
30 selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton,
sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive,

- 67 -

cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

59. A transgenic plant according to claim 57, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

60. A transgenic plant according to claim 57, wherein the plant is a monocot.

61. A transgenic plant according to claim 57, wherein the plant is a dicot.

15

62. A transgenic plant according to claim 57, wherein there are at least 2 coupled domains, each from a different source organism.

20

63. A transgenic plant according to claim 57, wherein there are 3 or more coupled domains.

64. A transgenic plant seed transformed with the nucleic acid molecule of claim 49.

25

65. A transgenic plant seed according to claim 64, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

30

- 68 -

66. A transgenic plant seed according to claim 64, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 67. A transgenic plant seed according to claim 64, wherein the plant is a monocot.

68. A transgenic plant seed according to claim 64, wherein the plant is a dicot.

10

69. A method of imparting disease resistance to plants comprising: applying a protein according to claim 47 to a plant or a plant seed under conditions effective to impart disease resistance to the plant or to a plant grown from the plant seed.

15

70. A method according to claim 69, wherein the protein is applied to a plant.

71. A method according to claim 69, wherein the protein is applied to a plant seed and further comprising: planting the plant seed under conditions effective to impart disease resistance to a plant grown from the plant seed.

20

72. A method of enhancing plant growth comprising: applying a protein according to claim 47 to a plant or a plant seed under conditions effective to enhance growth of the plant or of a plant grown from the plant seed.

25

73. A method according to claim 72, wherein the protein is applied to a plant.

30

74. A method according to claim 72, wherein the protein is applied to a plant seed and further comprising:
planting the plant seed under conditions effective to enhance growth of a plant grown from the plant seed.

5

75. A method of controlling insects comprising:
applying a protein according to claim 47 to a plant or a plant seed under conditions effective to control insects.

10

76. A method according to claim 75, wherein the protein is applied to a plant.

15

77. A method according to claim 75, wherein the protein is applied to a plant seed and further comprising:
planting the plant seed under conditions effective to grow a plant from the plant seed and to control insects.

20

78. A method of imparting stress resistance to plants comprising:
applying a protein according to claim 47 to a plant or a plant seed under conditions effective to impart stress resistance to the plant or to a plant grown from the plant seed.

25

79. A method according to claim 78, wherein the protein is applied to a plant.

30

80. A method according to claim 78, wherein the protein is applied to a plant seed and further comprising:
planting the plant seed under conditions effective to impart stress resistance to a plant grown from the plant seed.

81. A method of imparting disease resistance to plants comprising:

- 70 -

providing a transgenic plant or transgenic plant seed containing the nucleic acid according to claim 49 and

5 planting the transgenic plant or transgenic plant seed under conditions effective to impart disease resistance to the plant or to a plant grown from the plant seed.

82. A method according to claim 81, wherein a transgenic plant is provided.

10 83. A method according to claim 81, wherein a transgenic plant seed is provided.

84. A method of enhancing growth of plants comprising:
providing a transgenic plant or transgenic plant seed containing the
15 nucleic acid according to claim 49 and
planting the transgenic plant or transgenic plant seed under conditions effective to enhance growth of the plant or of a plant grown from the plant seed.

85. A method according to claim 84, wherein a transgenic plant is
20 provided.

86. A method according to claim 84, wherein a transgenic plant seed is provided.

25 87. A method of controlling insects comprising:
providing a transgenic plant or transgenic plant seed containing the nucleic acid according to claim 49 and
planting the transgenic plant or transgenic plant seed under conditions effective to control insects on the plant or on a plant grown from the plant seed.

30 88. A method according to claim 87, wherein a transgenic plant is provided.

89. A method according to claim 87, wherein a transgenic plant seed is provided.

5 90. A method of imparting stress resistance to plants comprising:
providing a transgenic plant or transgenic plant seed containing the
nucleic acid according to claim 49 and
planting the transgenic plant or transgenic plant seed under conditions
effective to impart stress resistance to the plant or to a plant grown from the plant
10 seed.

91. A method according to claim 90, wherein a transgenic plant is provided.

15 92. A method according to claim 90, wherein a transgenic plant seed is provided.

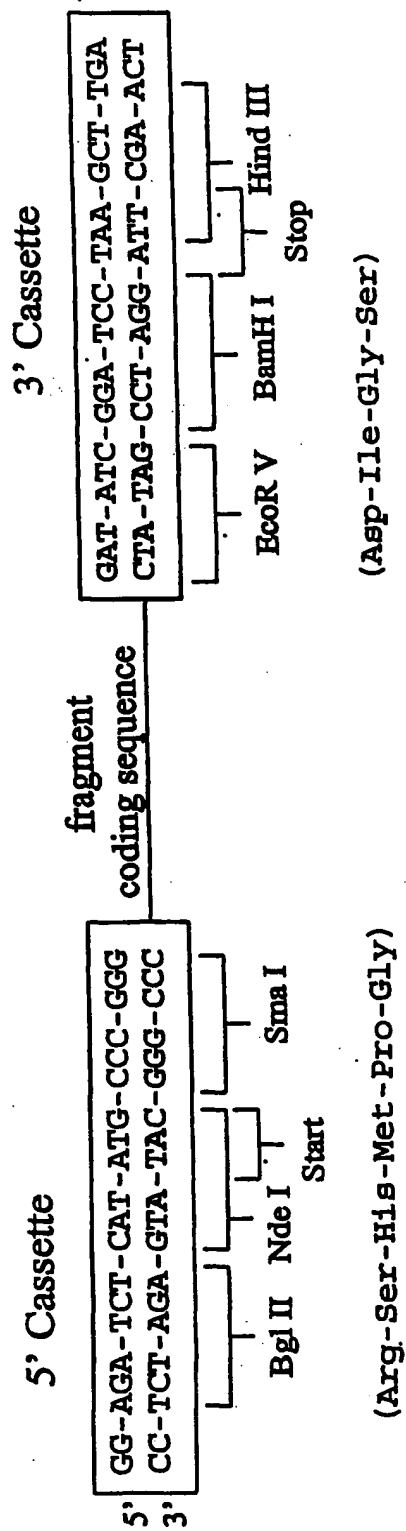


Figure 1

SEQUENCE LISTING

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THEREOF

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<151> 2000-06-16

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<170> PatentIn Ver. 2.1

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<210> 8

<211> 1838

<212> PRT

<213> Erwinia amyli vora

<400> 8

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 20 25 30
 Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
 35 40 45
 Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
 50 55 60
 Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80
 Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
 85 90 95
 Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
 100 105 110
 Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
 115 120 125
 Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
 130 135 140
 Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
 145 150 155 160
 Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
 165 170 175
 Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
 180 185 190
 Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
 195 200 205
 Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
 210 215 220
 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
 225 230 235 240
 Leu His Gln Gln Arg L u Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
 245 250 255
 Pr Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys

260 265 270
 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
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 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
 290 295 300
 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
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 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
 325 330 335
 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
 340 345 350
 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
 355 360 365
 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
 370 375 380
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
 385 390 395 400
 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
 405 410 415
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
 420 425 430
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
 435 440 445
 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
 450 455 460
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
 465 470 475 480
 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
 485 490 495
 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
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 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg

515 520 525
 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
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 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
 545 550 555 560
 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
 565 570 575
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 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
 595 600 605
 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
 610 615 620
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
 625 630 635 640
 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
 645 650 655
 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
 660 665 670
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
 675 680 685
 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
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 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
 705 710 715 720
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 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
 740 745 750
 Phe Ser L u Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
 755 760 765
 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly

770 775 780
 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
 785 790 795 800
 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
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 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
 820 825 830
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
 835 840 845
 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
 850 855 860
 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
 865 870 875 880
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
 885 890 895
 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
 900 905 910
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 915 920 925
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
 930 935 940
 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
 945 950 955 960
 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
 965 970 975
 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
 980 985 990
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 1060 1065 1070
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085
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 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
 1105 1110 1115 1120
 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135
 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165
 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
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 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
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 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
 1220 1225 1230
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
 1235 1240 1245
 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
 1250 1255 1260
 Ala Lys Lys Leu Lys Asn Thr Leu L u S r Leu Asp Ser Gly Glu Ser
 1265 1270 1275 1280
 Met Ser Phe S r Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val

1285	1290	1295
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Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly		
1315	1320	1325
Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile		
1330	1335	1340
Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys		
1345	1350	1355
Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile		
1365	1370	1375
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly		
1380	1385	1390
Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro		
1395	1400	1405
Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu		
1410	1415	1420
Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr		
1425	1430	1435
Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn		
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Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser		
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Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg		
1475	1480	1485
Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn		
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Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala		
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Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly		
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 1555 1560 1565
 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
 1570 1575 1580
 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
 1585 1590 1595 1600
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
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 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
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 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
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 1650 1655 1660
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
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 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
 1685 1690 1695
 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
 1700 1705 1710
 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
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 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
 1730 1735 1740
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
 1745 1750 1755 1760
 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
 1765 1770 1775
 Leu L u Gly Thr Ser Asn Ser Ala Ala Met Ser M t Glu Arg Asn Ile
 1780 1785 1790
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pr Arg Arg

1795 1800 1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
 1810 1815 1820

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 <211> 420
 <212> DNA
 <213> *Erwinia amylovora*

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 gctgaccac aaacttcaat aaccctgtat tcgatgctat tacagctgaa ttttgaaatg 240
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 cagcagtcgc tggagcatct ggatgaagca agttttagcg atatcgtag cggttcac 360
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<210> 10
 <211> 139
 <212> PRT
 <213> *Erwinia amylovora*

<400> 10
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Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
 35 40 45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
 50 55 60

Thr S r Il Thr Leu Tyr Ser Met Leu L u Gln Leu Asn Phe Glu Met
 65 70 75 80

Ala Ala M t Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe 11
 100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
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Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
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<210> 11

<211> 341

<212> PRT

<213> Pseudomonas syringae

<400> 11

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 20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
 65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
 100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
 130 135 140

Leu Asn Lys Ile Ala Gln Phe M t Asp Asp Asn Pro Ala Gln Phe Pro
 145 150 155 160

Lys Pro Asp Ser Gly S r Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
 195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
 210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
 225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
 245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
 260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
 275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335

Asn Gln Ala Ala Ala
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<210> 12

<211> 1026

<212> DNA

<213> *Pseudomonas syringae*

<400> 12

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 aagccggact cgggctcctg ggtgaacgaa ctcaaggaa acaacttcct tgatggcgac 540
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<210> 13

<211> 1729

<212> DNA

<213> *Pseudomonas syringae*

<400> 13

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 cctctgagtg cgggtcggag caataccagt ctccctgctg gcgtgtgcac actgagtcgc 180
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<210> 14

<211> 424

<212> PRT

<213> *Pseudomonas syringae*

<400> 14

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Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
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Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
 35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
 50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
 65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
 85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
 100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
 115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr
 130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
 145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
 165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
 180 185 190

Pro Gln Il Thr Pr Gln L u Ala Asn Pro Asn Arg Thr Ser Gly Thr
 195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile

210 215 220
 Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
 225 230 235 240
 Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
 245 250 255
 Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
 260 265 270
 Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
 275 280 285
 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
 290 295 300
 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
 305 310 315 320
 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
 325 330 335
 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
 340 345 350
 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
 355 360 365
 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
 370 375 380
 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
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 Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
 405 410 415
 Ala Ser Thr Gln His Thr Glu Leu
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<210> 15

<211> 344

<212> PRT

<213> Pseudomonas lanacearum

<400> 15

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 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45
 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
 65 70 75 80
 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
 85 90 95
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
 100 105 110
 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125
 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 Gln Gly Gly L u Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln 11
 260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met
 340

<210> 16

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 16

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 gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180
 ggcaacaccg gtaacaccgg caacgcgccc gcgaaggacg gcaatgccaa cgcggggcgcc 240
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<210> 17

<211> 10

<212> PRT

<213> Xanthomonas campestris

<400> 17

Met Asp Gly Ile Gly Asn His Phe Ser Asn
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<210> 18

<211> 20

<212> PRT

<213> Xanthomonas campestris pv. pelargonii

<400> 18

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
 1 5 10 15

Leu Leu Ala Met
 20